D. Insect Resistance Management.

BACKGROUND

Corn expressing the Cry3Bb1 protein is intended to provide protection against certain species of the corn rootworm (CRW) including the western corn rootworm (*Diabrotica virgifera virgifera*), northern corn rootworm (*Diabrotica barberi*) and Mexican corn rootworm (*Diabrotica virgifera zea*). Prior to registering Cry3Bb1 corn, an acceptable insect resistance management (IRM) plan is necessary. Monsanto designed a plan intended to be scientifically valid for resistance risk mitigation and feasible for growers to understand, to implement and to comply with the IRM Program. Since Monsanto acknowledges that a robust and practical IRM plan will require time to develop, they are proposing a three-year interim plan. An interim plan was submitted by Monsanto because they believe growers need to be able to grow MON 863 corn for a period of time so that important information can be generated and growers can be provided an understanding of corn rootworm IRM requirements.

It should be noted that previous IRM assessments for Plant Incorporated Protectants (PIPs) needed to consider the potential for resistant organisms feeding upon the PIP affecting the performance of registered microbial Bt pesticides against those organisms. In the case of CRW, there are no registered microbial or PIP products for the control of this organism at this time. Likewise, cross-resistance to Cry proteins in other PIPs or microbial products is not an issue.

Monsanto submitted several documents in support of their proposed IRM plan. An IRM plan for MON 863 corn dated June 20, 2000 was submitted to the Agency (MRID No. 451568-05). This submission included information on dose, CRW biology, simulation models of resistance development, and grower surveys. Research reports and results of grower surveys were also included in the Appendices of the June 2000 submission. An amended IRM plan dated January 8, 2002 was submitted to the Agency for review (MRID No. 455770-01). The amended plan titled "An Interim Insect Resistance Management Plan for Corn Event MON 863: A Transgenic Corn Rootworm Control Product" was intended to supercede MRID No. 451568-05. Therefore, MRID No. 451568-05 was used for additional information and as reference material, but was not formally reviewed. An additional preliminary research report dated February 20, 2001 was submitted to the Agency by Monsanto (MRID No. 453484-01). A review in the form of a data evaluation report (DER) of Monsanto's interim IRM plan found in MRID No. 455770-01 was reviewed by EPA in a memorandum from Robyn Rose to Mike Mendelsohn dated July 23, 2002 is attached to this memorandum.

Subsequent to EPA's final review of Monsanto's 3 year interim IRM plan, a FIFRA Scientific Advisory Panel (SAP) was convened in August 2002. The August 2002 SAP comments regarding Monsanto's interim IRM plan were documented in a memorandum from Paul Lewis to Marcia Mulkey dated November 6, 2002 (http://www.epa.gov/scipoly/sap/2002/August/august2002final.pdf). In response to the SAP, Monsanto submitted additional information to EPA in a document from Dennis Ward to Janet Andersen dated December 13, 2002. This additional information, along with additional clarifications provided to the Agency by Dr. Michael Caprio on December 20, 2002, Dr. David Andow on December 23, 2002 and Dr. Fred Gould on February 12, 2003 were incorporated into the final review and memorandum of February 14, 2003 from Robyn Rose to Mike Mendelsohn.

DISCUSSION

Pest Biology

In order to develop an appropriate IRM strategy for MON 863 corn, as well as all insect-protected transgenic crops, it is important to consider the biology of the target pest. Knowledge of pest biology is imperative in determining optimal size and placement of refuges that will encourage random mating between Bt and non-Bt corn. Based on the movement of CRW adults, a non-Bt corn refuge should be planted adjacent to or within MON 863 fields.

Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits and ovipositional habits) differ for WCRW and NCRW. WCRW and NCRW adults will feed on corn silks, pollen and young kernels in the ear tip; however, only WCRW feed on leaves. Since NCRW adults don't feed on corn leaves, they leave the field after pollination to find a younger field with pollen available (Branson and Krysan 1981). Since adult and larval CRW feed on various parts of the corn plant, both life stages may be exposed to the Bt protein and extended selection pressure may result (Meinke *et al.* 2001). Severe root damage from larval feeding will lead to plant lodging (where damaged corn stocks fall over making mechanical harvesting impossible) and yield losses.

WCRW and NCRW are univoltine, develop through one generation per year, in corn in most of the Corn Belt (Branson and Krysan 1981, Meinke *et al.* 2001). CRW typically oviposit where the adults are feeding which is almost exclusively in corn fields (Branson and Krysan 1981, Levine and Oloumi-Sadeghi 1991). In general, CRW adult emergence varies based on species, geography, weather, management practices such as insecticide use, population density and sex. For instance, males typically emerge before females and emergence, as well as fecundity, longevity and egg viability, are reduced in corn planted later in the season (Boetel and Fuller 1997, Levine and Oloumi-Sadeghi 1991, Meinke *et al.* 2001). It is unknown what effect corn rootworm protected transgenic corn will have on phenology, sex ratio and adult emergence patterns. Asynchronous adult emergence for Bt corn fields and non-Bt refuges may lead to nonrandom or assortive mating which may lead to an increased rate in the evolution of resistance. Nonrandom or assortive mating may also occur if Bt corn disrupts the synchrony of male and female CRW adult emergence (Meinke *et al.* 2001). Mating typically occurs within 24 to 48 hours of female adult emergence within the corn fields they emerged from or nearby (Meinke *et al.* 2001).

CRW larval movement is limited particularly in areas with low population densities (Meinke *et al.* 2001). Published and unpublished articles have reported varying distances that CRW larvae move. WCRW larvae may move from 12 to 16 inches and have been found in corn rows planted up to 40 inches apart (Suttle *et al*. 1967, Short and Luedtke 1970, Gray 1999). These studies suggest that CRW larvae hatching from eggs between rows are capable of finding and injuring corn roots regardless of row spacing. Since field corn is typically planted approximately 24 to 30 inches apart, CRW may move up to two rows according to current research. However, additional information is needed to verify the distance CRW larvae move within and between rows. In general, young CRW larvae (e.g., 1st, 2nd and sometimes 3rd instars) tend to move toward actively growing corn roots. Larval tendency toward respiring, growing corn roots is probably because of their ability to detect and move toward CO_2 (Strnad *et al.* 1986, Gray 1999). Young larvae will feed on the distal portion of corn roots and move through the soil to feed on new, short roots as they develop into later instars (Strnad and Bergman 1987, Gray 1999). It is therefore possible that a RS heterozygous larvae with a partially recessive resistance trait will begin feeding on transgenic corn roots and finish its development on adjacent non-transgenic roots which would result in a non-lethal dose of MON 863 and potential resistance.

NCRW and WCRW mated adults may be very mobile and have potentially high dispersal capabilities (Meinke *et al.* 2001). However, local dispersal is more common and involves movement within or among adjacent fields; whereas, migratory dispersal over long distances occurs in a small portion of populations and usually involves females (Meinke *et al.* 2001). Dispersal capabilities of the WCRW are greater than the NCRW. The WCRW is also a greater competitor and displaced the NCRW in Nebraska by 1980 (Hill and Mayo 1980). WCRW post-mating dispersal may be local or migratory. Published data suggests that some WCRW females may leave the field after mating to oviposit elsewhere (Coates *et al.* 1986). While sustained flights by mated female CRW are possible, movement by unmated females is limited. Knowledge of the maximum and average distance an adult CRW moves is limited. Additional research regarding adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges.

Additional information is needed on various aspects of CRW pest biology as it relates to a long-term IRM strategy. Characteristics of pest biology that are relevant to IRM (e.g., movement, feeding habits and ovipositional habits) differ for WCRW and NCRW; therefore, additional information on the biology of the WCRW and NCRW are needed. According the August 2002 SAP, the WCRW and MCRW are subspecies, therefore, a lot of the data collected on biology will relate to both species. However, the Panel concluded that data on adult mating behavior, male and female migration, and reproductive biology and fecundity of females is needed to determine if the IRM plan is suitable for MCRW. Although the SAP concluded that the same IRM strategy may be appropriate for the WCRW and NCRW, the Panel recommended additional research on the NCRW and the SAP suggested collecting data from several geographic locations of the WCRW. There are behavioral differences in WCRW populations from the western and eastern regions of their distribution so studies on aspects of pest biology such as movement should be conducted in several areas. Since the biology of the SCRW is very different from the other *Diabrotica* spp. and it is not adequately controlled by MON 863, the SCRW should not be considered.

Knowledge of the maximum and average distance adult and larval CRW movement is limited. In previous submissions (MRID Nos. 453484-01 and 455770-01), Monsanto listed and summarized studies currently underway that relate to the biology of CRW. Results of the these studies as well as additional research currently underway should be submitted to EPA after they are completed. Additional research regarding male and female (mated and unmated) adult and larval WCRW and NCRW dispersal potential is needed to determine placement of non-Bt corn refuges. In addition, more information is needed on mating habits, ovipositional patterns, number of times a female can mate and fecundity. The effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy also needs further investigation.

The August 2002 SAP identified several areas of additional research needed to fully understand CRW biology as it relates to an IRM strategy. The SAP concluded that male and female adult movement and fitness in MON 863 and non-transgenic corn should be evaluated in large-scale field studies. Data needed on movement include, but are not limited to, the distance males and females will move over time and the rate adults leave the natal field. The SAP also recommended an evaluation of "the impact of adult density on migration patterns of adults, whether a delay in male emergence from MON 863 affects male fitness and lowers their chances of mating, and whether there are sublethal effects of MON 863 on female fecundity, offspring quality and other fitness parameters." Data are also needed on the movement of NCRW male and female adults since little is currently known.

The NCR-46 (a technical committee consisting of research and extension CRW specialists and other cooperators) submitted a letter dated May 29, 2001 to the EPA that outlines additional CRW biology research. The August 2002 SAP recommended that the EPA consider the recommendations made by the NCR-46.

Dose

Identifying the level of dose, as related to selection intensity, is crucial when determining size and structure of a refuge needed to delay CRW resistance to MON 863 corn. CRW feeding behavior and survival and root expression data can be used to identify the dose of MON 863. From data currently available it can be concluded that MON 863 corn does not provide a high dose for CRW control. The August 2002 SAP suggested that it is not necessary to determine the difference between a low an moderate dose. It is adequate to differentiate between high dose and non-high dose products when determining effective refuge size. Therefore, MON 863 should be characterized as a non-high dose product.

According to the August 2002 SAP, comparing measures of fitness levels of susceptible homozygotes on MON 863 and non-Bt corn would provide a good approximation of selection intensity. The SAP suggested that the first step in approximating selection intensity would be to measure efficacy of MON 863 corn against CRW larvae. However, the Panel pointed out that selection intensity based on larval efficacy may be underestimated if sublethal effects or fitness costs occur. Selection intensity based on larval survival may also be underestimated if density dependent mortality is occurring. Resistant colonies of CRW should be developed to aid in determining selection intensity.

The SAP based their determination that MON 863 is a non-high dose product on the *SS* (homozygous susceptible) survival rate. The Panel also concluded that Monsanto's artificial diet assays had deficiencies, but were adequate to determine the LC_{50} for first instar larvae, level of larval resistance and dose.

Simulation Models of Resistance

In Monsanto's three year interim IRM plan, they requested planting a 20% non-Bt corn refuge to delay the potential of CRW resistance to Cry3Bb1. Monsanto's conclusion that a 20% refuge would be adequate to delay resistance to MON 863 corn was based on CRW biology, Cry3Bb1 effective dose, preliminary modeling results and agronomic considerations. It was concluded in the July 23, 2002 memorandum from Robyn Rose to Mike Mendelsohn that a 20% non-Bt corn refuge planted within or adjacent to MON 863 corn fields is expected to adequately delay the risk of CRW developing resistance to Cry 3Bb1. Monsanto's IRM interim plan and EPA's review of Monsanto's plan were addressed by the August 2002 SAP in the transmittal document from Paul Lewis to Marcia Mulkey dated November 6, 2002.

According to the SAP, the current models (Monsanto's modified Caprio model, Onstad *et al*. 2001 and Andow and Alstad 2002) show that the time to resistance does not substantially differ when the refuge size ranges from 10-25%. While the SAP agreed that resistance would not occur during an initial 3 years regardless of the size of the refuge, the majority of the Panel recommended a 50% refuge would be a desirable conservative approach since resistance would be delayed substantially longer. The SAP also stated that the amount gene frequency increases during an interim period is of greater importance than years to resistance because of the potential future impact on IRM. Since MON 863 is a non-high dose product, the Panel suggested that the potential for heritable quantitative variation and rapid evolution of resistance should be considered. In addition, the models only consider monogenic (single locus) resistance, but the SAP suggested that the models consider the potential for polygenic resistance in a non-high dose product.

Additional comments were made by the Panel regarding initial resistance allele frequency. Each of the models (Andow *et al*., Onstad *et al*., Monsanto's modified Caprio model) submitted in support of Monsanto's IRM plan designated the initial resistance allele frequency as .001.

However, the Panel suggested that the initial resistance allele frequency may be as low as 0.1 in a non-high dose product. Therefore, the Panel recommended that studies be conducted to determine if the initial resistance allele frequency is less than .01 and models should be run that investigate the full range of dominance values.

Monsanto responded to the August 2002 SAP in a submission letter from Dennis P. Ward (Monsanto Regulatory Affairs Manager) to Janet L. Andersen (Director, EPA's Biopesticides and Pollution Prevention Division) dated December 13, 2002. In the December 13 submission letter, Monsanto summarized results from four data sets from research they sponsored on the efficacy of MON 863. The first and third data sets consisted of field data collected from 1999 to 2002 by 22 scientists from 15 universities located in 15 states. The second data set included data collected by Dr. Bruce Hibbard (University of Missouri) and the fourth data set is from research conducted by Dr. Blair Seigfried (University of Nebraska). According to Monsanto, results of these four data sets demonstrate that the initial allele frequency is ≤.01. Detailed summaries of these four data sets will be submitted to the Agency for confirmation.

The first data set looked at 7500 corn plants artificially infested with ≥1200 CRW eggs/plant from naturally occurring populations. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 24 CRW/plant ($24 = 1200\,(1-(1-0.01)^2)$ would be resistant and the damage rating on the Iowa scale would be 3.1. Weiss *et al.* (1985) showed that <20 CRW = 3.1 on the Iowa scale. Since the average damage recorded in the first data set was 1.6, it can be concluded that the initial resistance allele frequency is ≤0.01.

The second data set summarized by Monsanto evaluated larval survival. In this study, ≥30 larvae were recovered per non-Bt corn plant at a wide range of egg infestation rates. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant larvae ($0.6 = 30(1-(1-0.01)^2)$ would occur per MON 863 corn plant. Since an average of 0.7 larvae were recovered (but not feeding normally), a ≤.01 initial resistance allele frequency can be assumed.

The third data set evaluated the number of surviving adult CRW. This data set includes several studies that infest corn plants with over 1200 eggs. Of the 1200 eggs, an average of 30 adults survived on non-transgenic corn. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 0.6 resistant adults ($0.6 = 30*(1-(1-0.01)^2)$) would occur per MON 863 plant and the damage rating on the Iowa scale would equal 3. Since damage averages 1.6 on the Iowa scale, a ≤.01 initial resistance allele frequency can be assumed.

The final data set (#4) examined 11 field collected adult female CRW populations reared in the lab. Between 134 and 489 larvae per population were examined for susceptibility to Cry3Bb1. These larvae demonstrated less than 6-fold difference between the most and least susceptible populations which is similar to or less than populations of European corn borer and corn earworm in their susceptibilities to Cry1Ac and Cry1Ab. If the initial resistance allele frequency is 0.01 and Hardy-Weinberg is assumed, then 2% (>20) of the larvae assayed would be resistant.

Monsanto asserts that since no putatively resistant large larvae were recovered at high doses which suggests no larvae survived and there was low variation (lower than with lepidopterans); therefore, a ≤.01 initial resistance allele frequency can be assumed.

Products with a resistance allele frequency ≥.01 would not have enough efficacy to justify commercialization (Bourguet *et al.* 2002, Ferre' and Van Rie 2002). If the initial resistance allele frequency were 0.1, then the efficacy of the MON 863 corn would be so poor that it would not be a marketable product. At a 0.1 initial resistance allele frequency, damage would be greater than 4.6 on the Iowa root rating scale and 0.01 would result in a 3 Iowa rating. The economic threshold in corn is a 3 on the Iowa rating scale. Monsanto has demonstrated that the average damage rating is 1.6. Since MON 863 consistently provides enough protection to result in much less than a 3 root rating, it can be concluded that the initial resistance allele frequency is ≤.01 based upon product performance.

Monsanto modified Caprio's model to include an initial resistance allele frequency of .01 and submitted these results in their December 13, 2002 letter to Janet Andersen. Results of running this model showed that a 20% refuge would delay resistance for approximately 7-16 years (see Fig 2 on page 13 and Fig 3 on page 14 of Monsanto's December 13, 2002 submission). For this model, SS survival was set at 0.5 and RS survival set at 0.8 which is partial dominance. Based on data collected by Monsanto and cooperators, MON 863 has been shown to control an average of 50% of the homozygous susceptible (SS) CRW. Therefore, the SS survival was designated 0.5 in the modified Caprio model.

According to Monsanto RS survival (dominance) probably equals 0.7. Therefore, basing dominance on ≥0.8 would be considered a very conservative approach. Monsanto modeled RS survival to range from 0.5 to 1. If a RS survival of 1 (absolute worst case) were to occur and the initial resistance allele frequency is assumed to be .01, then resistance would be delayed for approximately 13 years with a 20% refuge (Fig 1 on page 8 of Monsanto's December 13, 2002 submission). If RS survival is designated 0.8, then resistance will occur in approximately 16 years. According to Dr. David Andow (University of Minnesota), RS survival ranges between 0.3 and 0.8 (personal communication with Andow on 12/23/02). Therefore, a likely case assessment would be to designate RS as 0.8 which suggests that 80% of the heterozygotes survive.

Monsanto also provided the Agency with additional runs of the modified Caprio model that included conservative parameters representing a worst case scenario. These additional models included initial resistance allele frequencies of .01 and .001, RS dominance values of 0.7 and 0.8 and SS survival ranging from 0.1 to 0.8. Results of the model incorporating these conservative input parameters (e.g., initial allele frequency = .01; RS dominance value = 0.8; SS survival = 0.1) suggested that CRW resistance to Cry3Bb1 will not occur for at least seven years assuming 100% MON 863 market penetration and 100% IRM compliance (Table 1).

Table 1. Predictions for MON 863 durability with a 20% refuge

SS Survival	RS Dominance	Allele Frequency = .01	Allele Frequency = .001
0.1	0.7	7 years	9 years
0.1	0.8	7 years	9 years
0.3	0.7	11 years	15 years
0.3	0.8	10 years	13 years
0.5	0.7	20 years	30 years
0.5	0.8	16 years	23 years
0.8	0.7	> 100 years	> 100 years
0.8	0.8	> 50 years	> 50 years

Monsanto also commented on the SAP's recommendation to consider polygenic resistance in the simulation models. According to Monsanto, results of the model will not differ if polygenic resistance is considered rather than monogenic resistance. Dr. Mike Caprio (Mississippi State University) agreed with Monsanto's conclusion. According to, Dr. Caprio applying monogenic or polygenic resistance to the models does not affect the outcome in the absence of refuge (personal communication with Mike Caprio on 12/20/02, Caprio 1998). Groeters and Tabashnik (2000) concluded "that the intensity of selection , rather than the number of loci conferring resistance, is central in determining rates of resistance evolution and effectiveness of refuges." This new information provided to the Agency by Monsanto after the August 2002 SAP suggests that assuming CRW resistance to MON 863 is polygenic rather than monogenic will not affect the results of the models.

Based on the additional information submitted to the Agency by Monsanto after the August 2002 SAP and results of running Caprio's modified model with a .01 initial resistance allele frequency, it can be concluded that a 20% refuge will delay resistance for approximately 7 to 16 years and probably longer since the model also assumes 100% adoption. However, Monsanto assumes that 50% of the susceptible homozygotes (SS) will be controlled. Efficacy data submitted thus far shows 17% to 62% larval survival on MON 863 corn. If the SS input parameter were changed to a lower level of efficacy (e.g., 0.3), then the years to resistance may decrease.

Based on the results presented in Monsanto's recent submission and recommendations from national experts, including the NCR 46, a 20% refuge should be adequate to delay resistance for 7 to 16 years. In addition, because growers are familiar with the 20% refuge required for currently registered Bt corn products, complianceis expected based on grower familiarity, feasability and presenting a consistent message to growers. A 20% refuge should be planted adjacent to or within fields. Additional research should be conducted during registration to support the continued use of a 20% refuge.

Refuge

A 20% non-Bt corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. There are two ways a grower can implement the refuge requirement. A non-Bt corn refuge can be planted as a continuous block adjacent to the MON 863 fields or as non-transgenic strips planted within transgenic field. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999, Meinke *et al.* 2001). If a 20% refuge is planted as row strips within a corn field, then at least 6 to 12 consecutive rows of non-Bt corn should be planted. (Onstad *et al.* 2001). This interim IRM plan is not intended for fields planted to increase inbred seed since these fields need to be isolated from external corn pollen sources. An in-field or adjacent non-Bt corn refuge would be inconsistent with inbred seed production practices.

Soil applied insecticides to control CRW larvae are acceptable on refuge acres. The ability to treat refuges with larval insecticides is necessary to avoid the potential for severe damage and economic impact. However, it is not acceptable to treat refuges for adult CRW control since these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically.

Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields then the non-Bt refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-Bt refuges should be planted on first year corn fields if the MON 863 hybrids are planted on first year corn fields.

Monitoring for Resistance

A resistance monitoring strategy for Bt corn is needed to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes (i.e., susceptibility changes) through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs. As such, the utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. Monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for *Diabrotica* spp. for their feasibility as resistance monitoring tools.

Grower participation (e.g., reports of unexpected damage) is an important step in resistance monitoring. Resistance monitoring is also important because it provides validation of biological parameters used in models. However, resistance detection/monitoring is a difficult and imprecise task. It requires both high sensitivity and accuracy. Good resistance monitoring should have well-established baseline susceptibility data so changes in pest susceptibility over time can be monitored. Although baseline susceptibility data is not completed at this time, research is being conducted to develop the baseline susceptibility of WCRW and NCRW to MON 863. These data are also needed for MCRW.

A comprehensive monitoring plan that targets the CRW and addresses when and where monitoring will occur is needed and should be developed within two years of commercialization. The August 2002 SAP recommended a two tiered approach to monitoring for CRW resistance to MON 863. The Panel recommended tier 1 monitoring methods should identify locations that would merit tier 2 laboratory bioassays. Early detection monitoring should be directed to areas with the highest rate of MON 863 adoption since these areas represent the highest risk of resistance occurring.

The August 2002 SAP suggested that current methods used for early detection of resistance probably do not have the necessary level of sensitivity. Therefore, the Panel recommended potential alternatives to the insect bioassay using artificial diet. For instance, susceptibility of neonate larvae to corn lines expressing varying levels of the Cry3Bb1 protein (e.g., events MON 863, MON 862, MON 853 and MON 854). Measuring larval mortality and growth data with various corn lines rather than artificial diet would be easier and may eliminate some of the problems associated with the feeding bioassay such as mold growth on the artificial diet. Susceptibility data should also be collected for the NCRW and MCRW.

The SAP also suggested that data on root damage may be used as a monitoring tool. However, a method of using root damage ratings to monitor for resistance has not been developed or validated at this time. It also may be possible to use data on emergence patterns in the MON 863 and non-Bt corn refuges. More females than males from susceptible populations tend to emerge from MON 863. It may be possible to evaluate the percentage of males emerging and be correlated with resistance.

Monitoring will become more important after the accrual of multiple growing seasons of exposure and grower adoption increases. In addition to baseline susceptibility data, information is needed to determine how many individuals need to be sampled and in how many locations. The chance of finding a resistant larvae in a Bt crop depend on the level of pest pressure, the frequency of resistant individuals, the location and number of samples that are collected, and the sensitivity of the detection technique. Therefore, as the frequency of resistant individuals or the number of collected samples increases, the likelihood of locating a resistant individual increases (Roush and Miller 1986). If the phenotypic frequency of resistance is one in 1,000, then more than 3,000 individuals must be sampled to have a 95% probability of one resistant individual (Roush and Miller 1986).

Remedial Action

The initial observation of unexpected CRW damage or suspected resistance will likely occur by the grower. Unexpected damage will probably be observed as lodged corn plants. Growers should be required to report any unexpected CRW damage such as lodged plants to the registrant. The August 2002 SAP identified the following four steps a registrant should take to determine if further testing is needed to confirm resistance is occurring.

1. "request the grower check planting records"
2. "rule out damage from nontarget insects, weather, or other environmental factors"
3. "conduct tests to verify MON 863 was planted and that the correct percentage of plants are expressing"
4. "if plants are MON 863 and damage approaching a 0.5 (node-injury scale) is found on any expressing plant, evaluate roots from the corresponding refuge"

Resistance should be confirmed by a standard diet bioassay or evaluation of root node injury. An insect diet bioassay with the Cry3Bb1 protein that results in a LC_{50} that exceeds the upper limit of the 95% confidence interval of the LC_{50} established from baseline measurements of susceptible populations could be used to confirm resistance. Alternatively, resistance may be confirmed when one or more root nodes of at least 50% of Cry3Bb1 plants grown in the laboratory are destroyed. A discriminating concentration bioassay may also be used to confirm resistance; however, this method may take a long time to develop. The August 2002 SAP also recommended investigating the potential of using samples of populations surviving on Bt corn or an evaluation of larval root tunneling to confirm resistance.

Confirmed resistance should be reported to EPA as soon as possible and must be within 30 days. Once resistance has been confirmed, alternative control measures to reduce or control the local target pest population should be recommended to customers, extension agents, consultants, university cooperators, seed distributors, processors, state regulatory authorities, EPA regional and national authorities, and any other pertinent personnel of the incidence(s) of resistance in the affected area. Where appropriate, customers and extension agents in the affected area should apply insecticides and/or crop rotation practices to control any potentially resistant individuals.

As soon as possible following confirmation of resistance, but must be within 90 days, Monsanto should notify the Agency of the immediate mitigation measures that were implemented and submit a proposed long-term resistance management action plan for the affected area. Monsanto should work closely with the Agency in assuring that an appropriate long-term remedial action plan for the affected area is implemented. A remedial action plan that is approved by EPA should be implemented that consists of some or all the following elements, as warranted: 1) Inform customers and extension agents in the affected area of pest resistance; 2) Increase monitoring in the affected area, and ensuring that local target pest populations are sampled on an annual basis; 3) Recommend alternative measures to reduce or control target pest populations in the affected area; 4) Implement intensified local IRM measures in the affected area based on the latest research results. The implementation of such measures will be coordinated by the Agency with other registrants; and 5) Monsanto should cease sales of all MON 863 Bt corn hybrids until resistance has been shown to have been abated. During the sales suspension period, Monsanto may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy should be coordinated with the Agency.

For the growing season(s) following a confirmed resistance incident(s), Monsanto should maintain the sales and distribution suspension of all MON 863 hybrids potentially affected by the resistant pest populations or areas in which resistance is considered to be serious. This must be done within the affected region or if undetermined, the affected county(ies) and proximate surrounding counties. This sales suspension should remain in place until resistance has been determined to have subsided (within 5 to 10% or one standard deviation of baseline levels). In addition, Monsanto should develop, recommend, and implement alternative resistance management strategies for controlling the resistant pest(s) on corn with all necessary personnel (e.g. growers, extension agents, consultants, seed distributors, processors, university cooperators, and state/federal officials) in the affected region/county(ies) and surrounding counties of the resistance situation. All necessary personnel (e.g. growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region/county(ies) and surrounding counties of the resistance situation should be informed. Monitoring and surveillance in the affected area(s) for resistance and define the boundaries of the affected region should be intensified and studies on the rate of decline of resistance in the field should be conducted. Monsanto should continue to work with the Agency, states, grower groups, extension agents, consultants, university cooperators, or other expert personnel and other stakeholders to insure the implementation and development of appropriate mitigation measures for resistance in the affected areas.

Grower Education and Compliance

Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that Bt fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. The 2000 SAP also suggested that a comprehensive education program may help increase IRM compliance (SAP 2001). Ideally, the educational messages presented to growers should be consistent (among different registrants if applicable for CRW) and reflect the most current resistance management guidelines. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, Internet sites, toll-free numbers for questions or further information, and educational publications.

To avoid confusing or discouraging growers, new IRM programs should be kept simple and consistent with existing programs so that growers will not be discourage from properly implement IRM or will not grow transgenic crops. Growers should be required to sign a technology use agreement that outlines IRM requirements and acknowledges the growers responsibility to comply with them on an annual basis. The agreement will also state that growers received the Product Use Guide. This agreement may be a section of the growers order sheet or some other document or format. An annual industry-supported survey conducted by a third party should be submitted to the Agency as a tool to monitor grower compliance. Additional education efforts should target non-compliant growers and access to the technology will be limited for growers found to be non-compliant.

COMMENTS

A number of comments were received in regards to the announcement of the proposed registration and in response to the SAP meeting.

It has been suggested that corn fields should be scouted the previous growing season and MON 863 should only be planted when economic thresholds are reached. The Agency encourages scouting fields and the use of economic thresholds for all pest management programs including those utilizing PIPs. However, CRW resistance to MON 863 is not expected to occur during the three year interim registration requested by Monsanto. Therefore, mandatory scouting or prescriptive use of MON 863 is not necessary at this time. In addition, the potential for cross-resistance is not an issue for MON 863 since no microbial pesticides or PIPs are currently registered for corn with the same mode of action as Cry3Bb1 and CRW is not a listed pest on any current Bt microbial products

The NCR-46 committee which consists of research and extension CRW specialists as well as other cooperators commented on Monsanto's interim IRM plan at the request of the BPPD IRM Team. This group is recognized as the national authorities on CRW biology, ecology and management. The NCR-46 agrees that the potential for CRW to develop resistance to MON 863 during a three year interim period with low adoption rates is negligible. Since there is not a high dose of MON 863, there will be a significant number of CRW larvae survival. In addition, evidence of changes in larval feeding behavior on MON 863 corn will lead to increased larval survivorship. This increase in larval survivorship will result in a decreased chance of resistance.

Additional comments received by the Agency recommended limiting sales of MON 863 (e.g., maximum of 25% MON 863 grown per county). However, the 2002 SAP suggested limiting sales of MON 863 for IRM purposes to an on-farm level rather than by region, state or county. The Agency concludes that additional acreage limitations of MON 863 beyond the 20% refuge requirement are not necessary during the three interim period requested by Monsanto since market penetration is expected to be low relative to the potential market share at full maturity. According to Monsanto projections, MON 863 corn adoption will be similar to Roundup Ready® corn which had approximately 1% (790,000 acres), 2.5% (2 million acres) and 5% (4 million acres) market penetration during the first three years of commerical use. In addition, the Agency has predicted that acreage will be limited during the three year interim registration due to technology fees and grower practices. With any new technology, initial grower adoption rates are initially low and increase over time. Effectiveness of the technology, information dissemination, hybrid availability and grower characteristics influence the growers level of awareness, interest, evaluation, trial and eventual adoption of MON 863 corn. Growers will also probably utilize other CRW control techniques that will limit adoption of MON 863 over time particularly as seed treatments and other transgenic products become available. In addition, models have predicted that a $15 technology fee will result in 43% adoption of MON 863 corn in the year 2013; therefore, less than 43% adoption is expected during the initial three years of commercialization.

Seed mixes were also mentioned as a consideration. A refuge strategy that utilizes a seed mix would include 20% non-Bt corn randomly mixed with 80% MON 863 corn. The SAP recommended investigation of a "seed mix impact on selection intensity"; however, the Agency recognizes that seed mix refuges may not be practical. Since the Bt corn should not be treated with insecticides to control CRW larvae, the non-Bt corn plants that are randomly planted throughout the MON 863 field will not be protected from damage. This may lead to a destruction of non-Bt corn plants and a decrease in yield. In addition, CRW feeding damage leading to the destruction of non-Bt corn plants may result in a reduction in the effective refuge size. A seed mix is also not preferred because the Agency believes that IRM compliance will be greater if one consistent message is communicated to growers. Planting a Bt/non-Bt corn seed mix is not an acceptable refuge strategy for Bt corn currently grown to control lepidopteran pests. Recommending a seed mix as a refuge strategy for coleopteran-active Bt corn may mislead growers to conclude that a seed mix is acceptable for all Bt corn.

CONCLUSIONS

A 20% non-Bt corn refuge is sufficient for the 3 year interim period while additional information are being gathered. The non-Bt corn refuge should be planted as continuous blocks adjacent to the MON 863 fields, as perimeter strips or, as non-transgenic strips planted within transgenic field. A 20% non-Bt corn refuge is necessary to produce an adequate number of CRW susceptible to the Cry3Bb1 protein. Considering the limited movement of CRW larvae, planting refuges close to transgenic fields in large blocks is preferred to narrow strips (Gray 1999, Meinke *et al.* 2001). If a 20% refuge is planted as row strips within a corn field, then at least 6 to 12 consecutive rows should be planted 0.5 meters apart and Bt corn strips should be 9 to 18 meters from refuge rows (Onstad *et al.* 2001).

Seed and granular insecticide treatments to control CRW larvae are acceptable on refuge acres. However, it is not acceptable to treat refuges for adult CRW control these treatments may diminish the effectiveness of the refuge. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically. Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds. For example, if MON 863 hybrids are planted on continuous corn fields then the non-Bt refuge should be planted on continuous corn fields or both should be planted on first-year corn acres. Likewise, non-Bt refuges should be planted on first year corn fields if the MON 863 hybrids are planted on first year corn fields.

Additional research is needed to establish a long-term IRM strategy for MON 863 corn. The August 2002 SAP recognized areas of research recommended by the NCR 46 and identified ten additional areas needing further investigation for a basic scientific assessment. The NCR 46 identified the following topics requiring additional research in order to develop a long-term IRM plan.

- "Characterize tissue expression, dose, and the mechanism by which corn rootworms survive on transgenic corn expressing Cry3Bb.
- Continue to quantify movement patterns of corn rootworm larvae when feeding on transgenic (expressing Cry3Bb) and nontransgenic corn.
- Quantify pre- and post-mating dispersal of corn rootworm, movement within and between fields, and its implications for IRM.
- Quantify the relative fitness of rootworm individuals that survive on transgenic corn vs. nontransgenic corn.
- Re-evaluate the host status of major grassy cornfield weeds and other grasses commonly found near corn; estimate the potential impact these alternate hosts may have on corn rootworm population dynamics.
- Continue to develop toxicological bioassays and resistance monitoring techniques.
- Determine the genetic nature of resistance to corn rootworm-active Cry compounds.
- Improve rearing techniques for certain corn rootworm species to facilitate laboratory and greenhouse bioassays, genetic studies, etc.
- Generate more complete data sets on transgenic efficacy, adult emergence from transgenic corn, etc. for all targeted corn rootworm species.
- Evaluate IRM options other than a refuge strategy, especially if an event is not classified as high-dose.
- Examine the impacts of refuge configuration, including seed mixtures, on development of resistance and likelihood of farmer adoption.
- Continue to develop and refine computer simulation models that build on current knowledge to guide development of IRM strategies.
- Reconcile corn rootworm and ECB IRM needs into an optimal IRM plan."

In the November 6, 2002 memorandum from Paul Lewis to Marcia Mulkey, the August 2002 SAP identified the following areas needing further investigation.

- "What is the selection intensity on corn rootworm larvae from MON 863 in different regions/soils/moistures and at different densities?"
- "What is the selection intensity on corn rootworm male and female adults from MON 863?"

- "What is the selection progeny through maternal effects?"
- "What is the impact of using whole fields versus rows within fields as refuges on population dynamics and on percent of refuge beetles mating with resistant beetles from the Bt fields?"
- "How would use of a seed mix impact selection intensity?"
- "Are some of the surviving larvae on MON 863 more genetically tolerant of the Bt toxin than the general population?"
- "What could we learn from a quantitative genetic model?"
- "Is male/female movement different in different areas?"
- "Can we develop appropriate monitoring strategies?"
- "Can we develop appropriate mitigation strategies?"

In addition to the NCR 46 and SAP recommendations for additional research that are listed above, MON 863 affect on CRW fitness should be investigated. Additional information is also needed on CRW biology, monitoring for resistance and mitigation/remedial action. These areas are covered in detail above as well as in the July 23 2002 memorandum from Robyn Rose to Mike Mendelsohn that includes a preliminary review of Monsanto's three year interim IRM strategy.

RECOMMENDATIONS

1. A 20% non-Bt corn refuge should be planted adjacent to or within MON 863 corn fields during the interim three year registration requested by Monsanto. If row strips within a corn field are implemented, then at least 6, and preferably 12 consecutive rows of non-Bt corn should be planted. Refuge acres may be treated to control CRW larvae with chemical insecticides, but insecticides should not be used on refuges to control CRW adults. Alternate hosts and seed mixes should not be utilized as refuges for CRW in MON 863 corn.
2. If growers spray their corn fields with insecticides to control pests other than CRW, then all acres (Bt and non-Bt) should be treated identically. Bt fields and the non-Bt refuge acres should be treated with identical agronomic practices such as irrigating all corn (Bt and non-Bt) at the same time. To ensure the production of similar numbers of CRW, Bt and non-Bt corn should be planted in fields with similar backgrounds.
3. Additional information is needed on various aspects of CRW pest biology as it relates to a long-term IRM strategy. More information is needed on adult and larval movement and dispersal, mating habits, ovipositional patterns, number of times a female can mate and fecundity. Additional data outlined in the pest biology section and conclusion sections below are needed to confirm the appropriateness of Monsanto's proposed IRM strategy for MON 863 field corn. All current and future research that addresses CRW biology and MON 863 IRM should be submitted to EPA.
4. Additional research is needed to determine if IRM strategies designed for WCRW and NCRW are appropriate for MCRW
5. The mechanism of potential resistance of CRW to MON 863 should be determined to

develop an appropriate long-term IRM strategy. Resistant colonies of WCRW, NCRW and MCRW should be developed to aid in determining selection intensity.

6. The effect of WCRW ovipositing in soybean prior to overwintering and extended diapause in NCRW on an IRM strategy needs further investigation.
7. Detailed summaries of the four data-sets identified in Monsanto's December 13, 2002 letter should be submitted to the Agency in confirmation of the conclusion that the initial resistance allele frequency is $\leq .01$.
8. A long-term resistance monitoring strategy for CRW-protected Bt corn is needed to test the effectiveness of resistance management programs. Baseline susceptibility studies currently underway should be continued for WCRW and initiated for NCRW and monitoring techniques such as discriminating dose concentration assays need to be thoroughly investigated for their feasibility as resistance monitoring tools.
9. Expected and confirmed resistance should be determined by the methods outlined in the monitoring section below. A remedial action plan is also necessary. If resistance is confirmed, all acres (Bt fields and non-Bt refuges) should be treated with insecticides targeted at CRW adults as well as larvae.
10. Growers should be required to sign a technology use agreement that outlines IRM requirements and acknowledges the growers responsibility to comply with them on an annual basis. The agreement should also state that growers received the Product Use Guide.

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